AMENDMENTS TO THE SPECIFICATION

On page 1, before the heading "FIELD OF THE INVENTION" please add the following paragraph:

-- This application is the National Stage of International Application No. PCT/EP03/04063 filed on April 17, 2003. --

On page 1, at line 13, before the heading "BACKGROUND OF THE INVENTION" please add the following paragraph:

-- A Sequence Listing, on Compact Disc, containinbg the text file entitled "MER133SEQLIST.TXT", created on October 17, 2004, and having a file size of _____ bytes, is incorporated herein by reference. --

Please replace the paragraph beginning at line 18 on page 10 of the specification with the following amended paragraph:

-- · an accordingly specified molecule, wherein said peptide sequences are selected from the group of peptides a-k below wherein;

a) = ILLFAVFDEGKSWHS (P1; SEQ ID NO: 74),

b) = SYKSQYLNNGPQRIG (P2; SEQ ID NO: 75),

c) = GPQRIGRKYKKVRFM (P3; SEQ ID NO: 76),

d) = YKWTVTVEDGPTKSD (P4; SEQ ID NO: 77),

e) = ASNIMHSINGYVFDS (P5; SEQ ID NO: 78),

f) = VAYWYILSIGAQTDF (P6; SEQ ID NO: 79),

g) = MSSSPHVLRNRAQSG (P7; SEQ ID NO: 80),

h) = CNIQMEDPTFKENYR (P8; SEQ ID NO: 81),

i) = STLFLVYSNKCQTPL (P9; SEQ ID NO: 82),

j) = ISQFIIMYSLDGKKW (P10; SEQ ID NO: 83),

k) = IARYIRLHPTHYSIRSTLRM (P11; SEQ ID NO: 84); -- ·

Please replace the paragraph beginning at line 4 on page 14 of the specification with the following amended paragraph:

-- · A particularly preferred FVIII species of molecule is one in which the B-domain of the molecule is lacking and yet which also comprises one or more amino acid substitutions in any of the remaining domains to result in the elimination from the sequence of one or more T-cell epitopes. A therapeutic B-domain deleted FVIII molecule of 1438 amino acid residues has been the subject of successful clinical trials as described by Lusher et al and references therein [Lusher, J.M. et al (2003) ~~*Haemophilia* 9: 38-49~~ *Haemophilia* 9: 38-49]. The amino acid sequence of such a protein is depicted as ~~FIGURE 9~~ FIGURE 10 (SEQ ID NO: 73) herein. --

Please replace the paragraph beginning at line 28 on page 16 and extending through line 8 on page 17 of the specification with the following amended paragraph:

-- This approach has proven to be a particularly effective method and is disclosed herein as an embodiment of the invention. The method can be applied to test part of the sequence, for example a FVIII protein lacking all or part of the B-domain; the method can be applied to selected regions of the sequence, for example a sub-set of FVIII peptides such as all or some of those listed in FIGURE 1; or the method may be applied to test whole FVIII sequence. In the present studies, the method has involved the testing of overlapping FVIII-derived peptide sequences in a scheme so as to scan and test a FVIII sequence (SEQ ID NO: 73, FIGURE 10) lacking the B-domain. The synthetic peptides are tested for their ability to evoke a proliferative response in human T-cell cultured *in vitro*. Where this type of approach is conducted using naïve human T-cells taken from healthy donors, the inventors have established that in the operation of such an assay, a stimulation index equal to or greater than 2.0 is a useful measure of induced proliferation. The stimulation index is conventionally derived by division of the proliferation score (e.g. counts per minute of radioactivity if using ^{3}H-thymidine incorporation) measured to the test peptide by the score measured in cells not contacted with a test peptide. --

Please replace TABLE 1 bridging pages 18 and 19 of the specification with the following amended table:

--

Peptide Sequence	Residue #*
ATRRYYLGAVELSWD	1 - 15
SWDYMQSDLGELPVD	13 - 27
PVDARFPPRVPKSFP	25 - 39
SVVYKKTLFVEFTDH	43 - 57
VEFTDHLFNIAKPRP	52 - 68
PWMGLLGPTIQAEVY	67 - 81
LKNMASHPVSLHAVG	88 - 102
VSYWKASEGAEYDDQ	103 - 117
PGGSHTYVWQVLKEN	130 - 144
ASDPLCLTYSYLSHV	148 - 162
ILLFAVFDEGKSWHS	196 - 210
SLPGLIGCHRKSVYW	241 - 255
VYWHVIGMGTTPEVH	253 - 267
QASLEISPITFLTAQ	283 - 297
YIAAEEEDWDYAPLV	385 - 399
SYKSQYLNNGPQRIG	406 - 420
GPQRIGRKYKKVRFM	415 - 429
PYNIYPHGITDVRPL	472 - 496
YKWTVTVEDGPTKSD	511 - 252
KESVDQRGNQIMSDK	556 - 570
QIMSDKRNVILFSVF	565 - 579
PAGVQLEDPEFQASN	598 - 612
ASNIMHSINGYVFDS	610 - 624
LQLSVCLHEVAYWYI	625 - 639
VAYWYILSIGAQTDF	634 - 648
GAQTDFLSVFFSGYT	643 - 657
GCHNSDFRNRGMTAL	691 - 705
TGDYYEDSYEDISAY	712 - 726
LLSKNNAIEPRSFSQ	728 - 742

MSSSPHVLRNRAQSG	817 - 831
NEHLGLLGPYIRAEV	859 - 873
AWAYFSDVDLEKDVH	940 - 954
CNIQMEDPTFKENYR	1009 - 1023
IGEHLHAGMSTLFLV	1108 - 1122
STLFLVYSNKCQTPL	1117 - 1131
ISQFIIMYSLDGKKW	1204 - 1218
IARYIRLHPTHYSIR	1251 - 1265
RLHPTHYSIRSTLRM	1256 - 1270

* Sequence residue numbering according to B domain deleted sequence (~~FIGURE 9~~ SEQ ID NO: 73 in FIGURE 10) --

Please replace TABLE 2 on page 20 of the specification with the following amended table:

--

PeptideNo.	Residue # *	Peptide Sequence	Domain	SEQ ID NO:
P1	196 - 210	ILLFAVFDEGKSWSH	A1	74
P2	406 - 420	SYKSQYLNNGPQRIG	A2	75
P3	415 - 429	GPQRIGRKYKKVRFM	A2	76
P4	511 - 525	YKWTVTVRDGPTKSD	A2	77
P5	610 - 624	ASNIMHSINGYVFDS	A2	78
P6	634 - 648	VAYWYILSIGAQTDF	A2	79
P7	817 - 831	MSSSPHVLRNRAQSG	A3	80
P8	1009 - 1023	CNIQMEDPTFKENYR	A3	81
P9	1117 - 1131	STLFLVYSNKCQTPL	A3	82
P10	1204 - 1218	ISQFIIMYSLDGKKW	C1	83
P11	1251 - 1270	IARYIRLHPTHYSIRSTLRM	C1	84

* Sequence residue numbering according to B domain deleted sequence SEQ ID NO: 73 (~~FIGURE 9~~ FIGURE 10) --

Please replace the paragraph beginning at line 4 on page 21 of the specification with the following amended paragraph:

-- One example of such a set of preferred modifications is provided by the disruption of the T-cell epitope encompassed by peptide P10 with the sequence ISQFIIMYSLDGKKW (SEQ ID NO: 83; residues 1204-1218 of SEQ ID NO: 73). This epitope maps to the C1 domain of FVIII and is exemplary of an epitope able to evoke proliferation of *ex vivo* T-cells taken from both haemophiliac blood and the cells of a healthy non haemophiliac individual. --

Please replace the paragraph beginning at line 10 on page 21 of the specification with the following amended paragraph:

-- Guided by the results of *in silico* analysis of this peptide sequence with respect to its MHC class II ligand activity, site directed mutagenesis procedures have been applied to produce substitutions at residues F1207, I1208, I1209 and M1210. Residue numbering is according to the mature B-domain deleted FVIII sequence (FIGURE [[9]] 10; SEQ ID NO: 73). Mutagenesis at F1207 resulted in loss of FVIII expression and no detectable activity in the assays employed. In contrast active FVIII molecules were produced comprising substitutions I1208A or I1208T or I1208N and or I1209C and or M1210K or M1210N. Accordingly, active FVIII proteins with one or more of the above listed substitutions are preferred compositions under the scheme of the present. Particularly preferred substitutions are I1208A, I1209C, M1210K or M1210N and such substituted FVIII proteins are a further embodiment of the invention. --

Please replace the paragraph beginning at line 22 on page 21 of the specification with the following amended paragraph:

-- Similarly preferred embodiments comprise FVIII molecules containing substitutions within the T-cell epitope encompassed by peptide P8 with the sequence CNIQMEDPTFKENYR (SEQ ID NO: 81, residues 1009-1023 of SEQ ID NO: 73). This epitope maps to the A3 domain

of the FVIII protein. Site directed mutagenesis procedures have been applied to this sequence and the substitutions M1013K, I1011A or C or D or E or G or H or K or P or Q or R or S or T in SEQ ID NO: 73 have been established to provide a molecule with retained functional activity with respect to the coatest assay and immunological parameters *in silico* and immunological assays *in vitro*. FVIII molecules encompassing the substitutions M1013K, I1011A or C or D or E or G or H or K or P or Q or R or S or T in SEQ ID NO: 73 are accordingly further embodiments of the invention. --

Please replace the paragraph beginning at line 32 on page 21 and continuing through line 8 on page 22 of the specification with the following amended paragraph:

-- Similarly preferred embodiments comprise FVIII molecules containing substitutions within the T-cell epitope encompassed by peptide P7 with the sequence MSSSPHVLRNRAQSG (SEQ ID NO: 80, residues 817-831 of SEQ ID NO: 73). This epitope also maps to the A3 domain of the FVIII protein.. Substitution at position V823 in SEQ ID NO: 73 is considered an especially desired modification and is an embodiment of the invention. Site directed mutagenesis procedures have been applied to this sequence and the substitutions V823A, or D or E or G or H or N or P or S or T in SEQ ID NO: 73 have been established to provide a molecule with retained functional activity with respect to the coatest assay and immunological parameters *in silico* and immunological assays *in vitro*. FVIII molecules encompassing the substitutions V823A, or D or E or G or H or N or P or S or T in SEQ ID NO: 73 are accordingly further embodiments of the invention. --

Please replace the paragraph beginning at line 10 on page 22 of the specification with the following amended paragraph:

-- A further example of a T-cell epitope peptide sequence shown to be active in haemophiliac blood samples is provided by the peptide P11 with the sequence IARYIRLHPTHYSIRSTLRM (SEQ ID NO: 84, residues 1251-1270 of SEQ ID NO: 73). This epitope maps to the C1 domain of the FVIII protein and has been identified previously as a significant driver of FVIII inhibitor

production [Jacquemin, M. et al (2003) ~~*Blood* 101: 1351-1358~~ *Blood* 101: 1351-1358]. Substitutions at positions Y1254, I1255, L1257, Y1262, I1264, L1268 in SEQ ID NO: 73 are considered especially desired modifications and are an embodiment of the invention. --

Please replace the paragraph beginning at line 18 on page 22 of the specification with the following amended paragraph:

-- Further similarly preferred embodiments comprise FVIII molecules containing substitutions at positions L1119, F1120, L1121, V1122 and Y1123 of the epitope encompassed by peptide P9 (STLFLVYSNKCQTPL ; SEQ ID NO: 82, residues 1117-1131 of SEQ ID NO: 73) also substitutions at positions Y636, Y638, I637, L638 and I639 of the epitope encompassed by peptide P6 (VAYWYILSIGAQTDF ; SEQ ID NO: 79, residues 634-648 of SEQ ID NO: 73); substitutions at positions I613 and I617 of the epitope encompassed by peptide P5 (ASNIMHSINGYVFDS ; SEQ ID NO: 78, residues 610-624 of SEQ ID NO: 73); substitutions at positions V515 and V517 of the epitope encompassed by peptide P4 (YKWTVTVRDGPTKSD ; SEQ ID NO: 77, residues 511-525 of SEQ ID NO: 73); substitutions at position I419 of the epitope encompassed by peptide P3 (GPQRIGRKYKKVRFM ; SEQ ID NO: 76, residues 415-429 of SEQ ID NO: 73); substitutions at positions Y407, Y411 and L412 of the epitope encompassed by peptide P2 (SYKSQYLNNGPQRIG ; SEQ ID NO: 75, residues 406-420 of SEQ ID NO: 73) and substitutions at positions L197, L198, F199, V201 and F202 of the epitope encompassed by peptide P1 (ILLFAVFDEGKSWSH ; SEQ ID NO: 74, residues 196-210 of SEQ ID NO: 73). --

Please replace the paragraph beginning at line 24 on page 26 of the specification with the following amended paragraph:

-- **FIGURE 8** provides exemplary T-cell assay data showing mutant peptides with a stimulation index of < 2.0 under conditions whereby the wt sequence peptide shows a stimulation index >2.0. Peptide sequences and PBMC donor allotype data are tabulated. Peptides: WTP8 (SEQ

ID NO: 1144); P8K+A (SEQ ID NO: 1145); P8K+T (SEQ ID NO: 1146); C32 (SEQ ID NO: 1147). --

Please replace the paragraph beginning at line 28 on page 26 and continuing through line 4 on page 27 of the specification with the following amended paragraph:

-- **FIGURE 9** shows a representation of the results achieved using the software simulation of peptide MHC class II binding. Results are shown for 18 different HLA-DR allotypes, each vertical column indicates the binding for a single allotype. Panel A shows the binding profile detected for the wt FVIII sequence around peptide 7 (P7, SEQ ID NO: 80; residues 817-831 of SEQ ID NO: 73). The peptide 7 sequence is highlighted. Panel B shows the binding profile detected for a modified peptide 7 sequence containing the substitution $V_{823}A$ and demonstrates loss of a high affinity ligand for multiple allotypes. In each panel the predicted MHC binding is depicted by denoting the first residue of each 13mer MHC class II ligand. The intensity of binding is denoted H, M or L based on the calculated binding score for each ligand for each allotype as indicated. Physical binding studies have previously indicated that scores of <500,000 constitute a negligible binding interaction. --

Please replace the paragraph beginning at line 6 on page 27 of the specification with the following amended paragraph:

-- **FIGURE 10** shows the amino acid sequence and numbering for a wild-type (WT) B-domain deleted human FVIII sequence (SEQ ID NO: 73). Amino acids are depicted using single letter code. --

Please replace the paragraph at lines 7-12 on page 31 of the specification with the following amended paragraph:

-- Since a B domain deleted variant of ~~factor~~ Factor VIII was to be cloned, the cDNA was amplified from the cDNA in two halves. The 5' end of the mRNA was amplified using the following primers:

SEQ ID [[NO.]] NO: 1 GCATCGCGCGCTAGCAATAAGTC**ATG**CAAATAGAG
SEQ ID [[NO.]] NO: 2 GAAGCT**C**CT**A**GGTTCAATGGCATTGTTTTTACTCA --

Please replace the paragraph at lines 14-18 on page 31 of the specification with the following amended paragraph:

-- ~~Seq ID No. 1~~ SEQ ID NO: 1 contains two restriction enzyme sites to facilitate cloning plus 24 nucleotides of the ~~factor~~ Factor VIII mRNA sequence surrounding the ATG start codon (bold). ~~Seq ID No. 2~~ SEQ ID NO: 2 is complimentary to nucleotides 2420 to 2454 of the ~~factor~~ Factor VIII mRNA and contains two changed nucleotides at positions 2445 and 2448 (shown in bold) which introduce a new restriction enzyme site whilst leaving the protein sequence unaffected. --

Please replace the paragraph at lines 20-25 on page 31 of the specification with the following amended paragraph:

-- The 3' end of the ~~factor~~ Factor VIII gene was amplified using the following primer pair:

SEQ ID [[NO.]] NO: 3:
TGAGTCTTAAGCTAGCTAGATACCTAG**G**AGCTTCTCCCAAAACCCACCAGTCTTGAA
ACGCC
SEQ ID [[NO.]] NO: 4:
TACGTCTCGAGTCAGTAGAGGTCCTGTGCCTCGCA --

Please replace the paragraph at lines 27-33 on page 31 of the specification with the following amended paragraph:

-- ~~Seq ID No.~~ SEQ ID NO: 3 contains restriction enzyme site NheI to facilitate cloning plus nucleotides 2442 to 2457 of the ~~factor~~ Factor VIII mRNA fused to nucleotides 5140 to 5164. This primer therefore creates the junction between the heavy and light chains where almost the entire B domain is absent. This primer also contains the nucleotide changes at positions 2445 and 2448 (shown in bold) which create the new restriction enzyme site also found in ~~Seq ID No.~~ SEQ ID NO: 2. ~~Seq ID No.~~ SEQ ID NO: 4 contains the final 24 nucleotides of the ~~factor~~ Factor VIII coding sequence plus a restriction enyme site to facilitate cloning. --

Please replace the paragraph beginning at line 34 on page 31 and continuing through line 11 on page 32 of the specification with the following amended paragraph:

-- PCR reactions were done using Expand HiFi polymerase (Roche) using the supplied buffer containing $MgCl_2$ in a final volume of 50µl. The reactions were made up to 1x buffer containing 200µM of each dNTP, 50pmols of each primer (either ~~Seq ID No.~~ SEQ ID NO: 1 plus ~~No.~~ SEQ ID NO: 2 or ~~Seq ID No.~~ SEQ ID NO: 3 plus ~~No.~~ SEQ ID NO:4), 2.5units RnaseH, and 5µl of reverse transcriptase reaction mix. The reactions were incubated at 37°C for 30min. in order for the RNA in the RNA/cDNA hybrid to be degraded by the RnaseH in order to increase the efficiency of the PCR reaction. The reactions were then heated to 94 °C for 2min. for initial denaturation, followed by cycling for the following temperatures and times: 94 °C 30sec., 55 °C 30sec., 72 °C 2.5min. During the first annealing step the recommended amount of polymerase was added and the reaction cycled 20 times. During the annealing step following the 20th extension step, a second aliquot of polymerase was added and the reaction cycled a further 20 times. The reaction was then incubated at 72 °C for 10min to ensure complete polymerisation of all strands. --

Please replace the paragraph beginning at line 25 on page 32 and continuing through line 2 on page 33 of the specification with the following amended paragraph:

-- White colonies were picked and dispersed in 20µl water. A sample from each resuspended colony was analysed via PCR in a reaction volume of 20µl using Taq polymerase (Roche) with the supplied buffer. Reactions were made up in 1x buffer containing 200µM of each dNTP, 50pmols each primer being the standard M13 forward and reverse primers (SEQ ID [[Nos]] NO: 5 and SEQ ID NO: 6 herein), and 1µl resuspended colony. The reactions were then heated to 94°C for 2min. for initial denaturation, followed by cycling x20 for the following temperatures and times: 94 °C 30sec., 60 °C 30sec., 72 °C 2min. The reaction was then incubated at 72 °C for 10min to ensure complete polymerisation of all strands. --

Please replace the paragraph at lines 4-29 on page 33 of the specification with the following amended paragraph:

-- A 5µl sample of each reaction was separated on a 1% agarose gel. Samples containing bands at approx. 2300bp were deemed positive for the ~~factor~~ Factor VIII 5' half insert and those containing bands at approx. 2150bp were deemed positive for the ~~factor~~ Factor VIII 3' half insert. For each insert, eight colonies were each inoculated into 5ml liquid cultures of 2YT broth/100ug/ml ampicillin and grown overnight at 37°C with shaking. Plasmid was prepared from 1.5ml of each culture using a Qiagen mini-prep kit and the plasmids were sent to a contract sequencing facility for sequence determination using the following primers:

5' half clones:

SEQ ID [[No.]] NO: 5	CGCCAGGGTTTTCCCAGTCACGAC	(M13 forward)
SEQ ID [[No.]] NO: 6	AGCGGATAACAATTTCACACAGGA	(M13 reverse)
SEQ ID [[No.]] NO: 7	ATGATCAGACCAGTCAAAGG	(factor VIII mRNA nt573-592)
SEQ ID [[No.]] NO: 8	CAGGAAATCAGTCTATTGGC	(factor VIII mRNA nt975-994)
SEQ ID [[No.]] NO: 9	TGGGTACATTACATTGCTGC	(factor VIII mRNA nt1372-1391)
SEQ ID [[No.]] NO: 10	ACAGTGACTGTAGAAGATGG	(factor VIII mRNA nt1768-1787)
SEQ ID [[No.]] NO: 11	GTCTTCTTCTCTGGATATACC	(factor VIII mRNA nt2179-2199)

3' half clones:

SEQ ID [[No.]] NO: 5	CGCCAGGGTTTTCCCAGTCACGAC	(M13 forward)
SEQ ID [[No.]] NO: 6	AGCGGATAACAATTTCACACAGGA	(M13 reverse)
SEQ ID [[No.]] NO: 12	GAGTAGCTCCCCACATGTTC	(factor VIII mRNA nt5370-5361)
SEQ ID [[No.]] NO: 13	GTGCACTCAGGCCTGATTGG	(factor VIII mRNA nt5786-5767)
SEQ ID [[No.]] NO: 14	AGGTGTTTTTGAGACAGTGG	(factor VIII mRNA nt6187-6168)
SEQ ID [[No.]] NO: 15	GAGGAAATTCCACTGGAACC	(factor VIII mRNA nt6594-6575)
SEQ ID [[No.]] NO: 16	AATCTCTGCTTACCAGCATG	(factor VIII mRNA nt6993-6974) --

Please replace the paragraph at lines 7-19 on page 34 of the specification with the following amended paragraph:

-- pCF85 was digested with BssHII and the ends made flush with T4 DNA polymerase (NEB) in the presence of 100μM each dNTP. The reaction was then heated to 70°C for 10min and then digested with AvrII. The released 2.3kbp fragment was purified via agarose gel electrophoresis and cloned into pCLF83 which had been digested with Bst98I, flush ended as above, digested with AvrII and gel purified. Positive bacterial colonies were identified via PCR screening as above using primers ~~Seq ID No.~~ SEQ ID NO: 11 and ~~Seq ID No.~~ SEQ ID NO: 17 (ATCAGTAAATTCCTGGAAAAC [Factor VIII mRNA nt5448-5428]). These primers amplify a fragment across the junction between the two halves of the ~~factor~~ Factor VIII gene and therefore a product of approx. 570bp is seen only if the cloning has been successful. A positive colony was selected and termed pCLF8. This colony was grown and DNA prepared and sequenced. Correct junctional sequences were confirmed using primers ~~Seq ID No.~~ SEQ ID NO: 6 and ~~Seq ID No.~~ SEQ ID NO: 5. The junction between the 5' and 3' halves was also verified using primer ~~Seq ID No.~~ SEQ ID NO: 17. --

Please replace the paragraph beginning at line 21 on page 36 and continuing through line 9 on page 37 of the specification with the following amended paragraph:

-- Peptide 10 contains four hydrophobic residues which have the potential to be the primary anchor for interaction with MHC Class II molecules, F1207, I1208, I1209 & M1210 (numbering

according to mature B domain deleted sequence SEQ ID NO: 73). Therefore these four residues were targeted for mutation to residues other than those which can potentially be primary anchors. F1207 was changed to:A, H, K, N, Q and R; I1208 was changed to: A, T, D, N; I1209 was changed to: A, C, D, N, P; M1210 was changed to: A, K, N and Q. Mutagenesis was done via overlap PCR using established protocols well known to those skilled in the art. Peptide 10 lies within a fragment of the ~~factor~~ Factor VIII nucleotide sequence bounded by PspOMI and SphI restriction sites. PCR primers for amplification of these fragment were synthesised corresponding to sequences just outside this region (~~Seq ID No.~~ SEQ ID NO: 18 and ~~No.~~ SEQ ID NO: 19 below). For mutagenesis of F1207, internal primers were synthesised as described below:

SEQ ID ~~No.18:~~ NO: 18	GGACACATTAGAGATTTTCA	(gene nt.3463-3482)
SEQ ID ~~No.19:~~ NO: 19	CAGTAATCTGTGCATCTGAT	(gene nt.3949-3930)
SEQ ID ~~No.20:~~ NO: 20	CTGAGAGATGTAGAGGCT	(gene nt.3675-3658)
SEQ ID ~~No.21:~~ NO: 21	AGCCTCTACATCTCTCAG**gcc**ATCATCATGT	(F1207A)
SEQ ID ~~No.22:~~ NO: 22	AGCCTCTACATCTCTCAG**cac**ATCATCATGT	(F1207H)
SEQ ID ~~No.23:~~ NO: 23	AGCCTCTACATCTCTCAG**aag**ATCATCATGT	(F1207K)
SEQ ID ~~No.24:~~ NO: 24	AGCCTCTACATCTCTCAG**aac**ATCATCATGT	(F1207N)
SEQ ID ~~No.25:~~ NO: 25	AGCCTCTACATCTCTCAG**cag**ATCATCATGT	(F1207Q)
SEQ ID ~~No.26:~~ NO: 26	AGCCTCTACATCTCTCAG**cgc**ATCATCATGT	(F1207R) --

Please replace the paragraph beginning at line 11 on page 37 and continuing through line 6 on page 38 of the specification with the following amended paragraph:

-- PCR were done using Expand HiFi polymerase (Roche) using the supplied buffer containing $MgCl_2$ in a final volume of 50µl. The 5' fragment reaction contained 1x buffer containing 200µM of each dNTP, 50pmols of each primer (~~Seq ID No.~~ SEQ ID NO: 18 plus ~~No.20~~ SEQ ID NO: 20), 100ng pCIF8, and 2.5 units Expand polymerase. Six 3' fragment reactions were set up and contained 1x buffer containing 200µM of each dNTP, 50pmols of each primer (~~Seq ID No.~~ SEQ ID NO: 19 plus either ~~Seq ID No.~~ SEQ ID NO: 21, 22, 23, 24, 25 or 26), 100ng pCIF8 and 2.5units Expand polymerase. The reactions were then heated to 94°C for 2min. for initial denaturation, followed by cycling for the following temperatures and times: 94

°C 30sec., 50 °C 30sec., 72 °C 30sec. The reactions were cycled 20 times and then incubated at 72 °C for 10min to ensure complete polymerisation of all strands.
One half of each PCR was separated on a 1% agarose gel and the 5' fragment of 226bp and the six different 3' fragments of 292bp were excised from the gel and purified using a Qiagen Gel Extract kit. The 5'fragment was then joined to each of the 3'fragments as follows: six further PCRs were done as described above using Expand HiFi polymerase using as template 2µl of eluted 5' fragment with 2µl of each of the six eluted 3' fragments with primers ~~Seq ID No.~~ SEQ ID NO 18 and ~~Seq ID No.~~ SEQ ID NO 19. Half of each of these reactions were separated on a 1% agarose gel and the six bands of 486bp were purified as described above. Each PCR product was digested with restriction enzymes PspOMI and SphI in a total reaction volume of 50µl overnight at 37 °C. 4µg plasmid pCIF8 was similarly digested for 2h at 37 °C and half of the plasmid and PCR fragment digests were run through a 1% agarose gel. The vector band of 7.2kbp and the PCR fragments of 391bp were excised from the gel and purified as above into final volumes of 30µl each in water. 1µl of vector was ligated to 3µl of each of the six fragments in standard 10µl ligation reactions using T4 DNA ligase and supplied buffer (Invitrogen). The reactions were incubated at room temperature for 4h. 1µl of each ligation reaction was electroporated into 20µl electrocompetent *E. coli* strain XL1-Blue (Stratagene) as recommended by the supplier using a 0.1cm gap cuvette. The cells were resuspended and allowed to recover also as recommended by the supplier. 10µl and 100µl aliquots of the electroporated cells were plated out on LB agar plates containing 100µg/ml ampicillin and grown at 37 °C overnight. --

Please replace the paragraph beginning at line 8 on page 38 and continuing through line 8 on page 39 of the specification with the following amended paragraph:

-- Four colonies from each plated ligation were picked and grown up overnight in 5ml liquid culture in 2YT broth plus 100µg/ml ampicillin at 37°C with vigorous shaking. Plasmid DNA was prepared from 1.5ml of each culture using a Qiagen Mini-Prep kit. Samples of each plasmid were DNA sequenced using primer ~~Seq ID No.~~ SEQ ID NO: 18. One plasmid from each group

of four, with the correct sequence was selected and stored for analysis via transfection for activity and expression levels.

This same general procedure was followed for creating the mutations at I1208, I1209 and M1210 of SEQ ID NO: 73. The primers used for each amino-acid were as follows:

I1208:

SEQ ID ~~No.18:~~ NO: 18	GGACACATTAGAGATTTTCA	(gene nt.3463-3482)
SEQ ID ~~No.19:~~ NO: 19	CAGTAATCTGTGCATCTGAT	(gene nt.3949-3930)
SEQ ID ~~No.20:~~ NO: 20	CTGAGAGATGTAGAGGCT	(gene nt.3675-3658)
SEQ ID ~~No.27:~~ NO: 27	AGCCTCTACATCTCTCAGTTT**gcc**ATCATGT	(I1208A)
SEQ ID ~~No.28:~~ NO: 28	AGCCTCTACATCTCTCAGTTT**acc**ATCATGT	(I1208T)
SEQ ID ~~No.29:~~ NO: 29	AGCCTCTACATCTCTCAGTTT**gac**ATCATGT	(I1208D)
SEQ ID ~~No.30:~~ NO: 30	AGCCTCTACATCTCTCAGTTT**aac**ATCATGT	(I1208N)

I1209:

SEQ ID ~~No.18:~~ NO: 18	GGACACATTAGAGATTTTCA	(gene nt.3463-3482)
SEQ ID ~~No.19:~~ NO: 19	CAGTAATCTGTGCATCTGAT	(gene nt.3949-3930)
SEQ ID ~~No.20:~~ NO: 20	CTGAGAGATGTAGAGGCT	(gene nt.3675-3658)
SEQ ID ~~No.31:~~ NO: 31	AGCCTCTACATCTCTCAGTTTATC**gcc**ATGTATA	(I1209A)
SEQ ID ~~No.32:~~ NO: 32	AGCCTCTACATCTCTCAGTTTATC**tgc**ATGTATA	(I1209C)
SEQ ID ~~No.33:~~ NO: 33	AGCCTCTACATCTCTCAGTTTATC**gac**ATGTATA	(I1209D)
SEQ ID ~~No.34:~~ NO: 34	AGCCTCTACATCTCTCAGTTTATC**aac**ATGTATA	(I1209N)
SEQ ID ~~No.35:~~ NO: 35	AGCCTCTACATCTCTCAGTTTATC**ccc**ATGTATA	(I1209P)

M1210:

SEQ ID ~~No.18:~~ NO: 18	GGACACATTAGAGATTTTCA	(gene nt.3463-3482)
SEQ ID ~~No.19:~~ NO: 19	CAGTAATCTGTGCATCTGAT	(gene nt.3949-3930)
SEQ ID ~~No.20:~~ NO: 20	CTGAGAGATGTAGAGGCT	(gene nt.3675-3658)
SEQ ID ~~No.36:~~ NO: 36	AGCCTCTACATCTCTCAGTTTATCATCgccTATAGTC	(M1210A)
SEQ ID ~~No.37:~~ NO: 37	AGCCTCTACATCTCTCAGTTTATCATCaagTATAGTC	(M1210K)
SEQ ID ~~No.38:~~ NO: 38	AGCCTCTACATCTCTCAGTTTATCATCaacTATAGTC	(M1210N)
SEQ ID ~~No.39:~~ NO: 39	AGCCTCTACATCTCTCAGTTTATCATCcagTATAGTC	(M1210Q) --

Please replace the paragraph beginning at line 4 on page 40 of the specification with the following amended paragraph:

-- Comparison of the activity values and expression levels revealed that all changes to F1207 resulted in absence of expression and hence activity (all residue numbering based on SEQ ID NO: 73). Mutation of I1208 to A resulted in expression and activity similar to unmodified ~~factor~~ Factor VIII wheras changes to T and N resulted in 25% and 50% losses in activity. For I1209, only mutation to C allowed expression and the activity of this variant was similar to unmodified ~~factor~~ Factor VIII. All changes to M1210 resulted in expression of proteins with appreciable activity with K and N being indistinguishable from unmodified ~~factor~~ Factor VIII. Therefore I1208A, I1209C, M1210K and M1210N are useful mutations for the removal of the T cell epitope within peptide 10. --

Please replace the paragraph beginning at line 15 on page 40 of the specification with the following amended paragraph:

-- Peptide 8 contains two amino-acids which are potential primary anchors for binding to MHC Class II molecules: I1011 and M1013 (numbering according to mature B domain deleted sequence SEQ ID NO: 73). Therefore these two residues were targeted for mutation to residues other than those which can potentially be primary anchors. Comparison to the ~~factor~~ Factor VIII sequences of other species (dog, mouse and rat) revealed that in dog ~~factor~~ Factor VIII, M1013 is K. K was also shown to be the best replacement for M1210 for mutagenesis of peptide 10. Therefore M1013 was mutated to K alone and in combination with all amino-acids at I1011 which do not have the potential to form primary anchors (i.e. A, C, D, E, K, N, P, Q, R, S, T). --

Please replace the paragraph beginning at line 25 on page 40 and continuing through line 14 on page 41 of the specification with the following amended paragraph:

-- Mutagenesis was done via overlap PCR using established protocols well known to those skilled in the art. Peptide 8 lies within 490bp fragment of the ~~factor~~ Factor VIII nucleotide sequence bounded by PflM1 and PspOMI restriction sites. PCR primers for amplification of this fragment were synthesised corresponding to sequences just outside this region (~~Seq ID No.~~ SEQ ID NO: 40 and ~~No.~~ 41 below). For mutagenesis of M1013K plus I1011X in SEQ ID NO: 73, internal primers were synthesised and used as described below:

SEQ ID ~~No.40:~~ NO: 40	ATGAGACCAAAAGCTGGT	(gene nt.3026-3043)
SEQ ID ~~No.41:~~ NO: 41	AGGCATTGATTGATCCG	(gene nt.3559-3543)
SEQ ID ~~No.42:~~ NO: 42	ATTGCAGGGAGCCCTGCAGT	(gene nt.3087-3068)
SEQ ID ~~No.43:~~ NO: 43	ACTGCAGGGCTCCCTGCAAT**ATC**CA**G**a**ag**GAAGA	(M1013K)
SEQ ID ~~No.44:~~ NO: 44	ACTGCAGGGCTCCCTGCAAT**gcc**CA**G**a**ag**GAAGA	(I1011A, M1013K)
SEQ ID ~~No.45:~~ NO: 45	ACTGCAGGGCTCCCTGCAAT**tgc**CA**G**a**ag**GAAGA	(I1011C, M1013K)
SEQ ID ~~No.46:~~ NO: 46	ACTGCAGGGCTCCCTGCAAT**gac**CA**G**a**ag**GAAGA	(I1011D, M1013K)
SEQ ID ~~No.47:~~ NO: 47	ACTGCAGGGCTCCCTGCAAT**gag**CA**G**a**ag**GAAGA	(I1011E, M1013K)
SEQ ID ~~No.48:~~ NO: 48	ACTGCAGGGCTCCCTGCAAT**ggc**CA**G**a**ag**GAAGA	(I1011G, M1013K)
SEQ ID ~~No.49:~~ NO: 49	ACTGCAGGGCTCCCTGCAAT**cac**CA**G**a**ag**GAAGA	(I1011H, M1013K)
SEQ ID ~~No.50:~~ NO: 50	ACTGCAGGGCTCCCTGCAAT**aag**CA**G**a**ag**GAAGA	(I1011K, M1013K)
SEQ ID ~~No.51:~~ NO: 51	ACTGCAGGGCTCCCTGCAAT**aac**CA**G**a**ag**GAAGA	(I1011N, M1013K)
SEQ ID ~~No.52:~~ NO: 52	ACTGCAGGGCTCCCTGCAAT**ccc**CA**G**a**ag**GAAGA	(I1011P, M1013K)
SEQ ID ~~No.53:~~ NO: 53	ACTGCAGGGCTCCCTGCAAT**cag**CA**G**a**ag**GAAGA	(I1011Q, M1013K)
SEQ ID ~~No.54:~~ NO: 54	ACTGCAGGGCTCCCTGCAAT**cgc**CA**G**a**ag**GAAGA	(I1011R, M1013K)
SEQ ID ~~No.55:~~ NO: 55	ACTGCAGGGCTCCCTGCAAT**agc**CA**G**a**ag**GAAGA	(I1011S, M1013K)
SEQ ID ~~No.56:~~ NO: 56	ACTGCAGGGCTCCCTGCAAT**acc**CA**G**a**ag**GAAGA	(I1011T, M1013K) --

Please replace the paragraph beginning at line 25 on page 41 of the specification with the following amended paragraph:

-- Comparison of expression levels and activity for the above mutants revealed that M1013K alone was very similar to unmodified ~~factor~~ Factor VIII (all residue numbering based on SEQ ID NO: 73). In addition, combination mutants containing M1013K plus I1011A, E, P, S or T were

also indistinguishable from unmodified ~~factor~~ Factor VIII. Therefore these mutations are useful for the removal of T cell epitopes associated with peptide 8. - -

Please replace the paragraph beginning at line 32 on page 41 and continuing through line 6 on page 42 of the specification with the following amended paragraph:

- - Peptide 7 (P7) contains one amino-acid which is a potential primary anchor for binding to MHC Class II molecules, V823 (numbering according to mature B domain deleted sequence SEQ ID NO: 73). Therefore this residue was targeted for mutation to residues other than those which can potentially be primary anchors (i.e. A, C, D, E, K, N, P, Q, R, S, T). Mutagenesis was done via overlap PCR using established protocols as previously. Peptide 7 lies within 766bp fragment of the ~~factor~~ Factor VIII nucleotide sequence bounded by restriction enzymes AvrII and PflM1. PCR primers for amplification of this fragment were synthesised corresponding to sequences just outside this region (~~Seq ID No.~~ SEQ ID NO: 57 and ~~No.~~ SEQ ID NO: 58 below). For mutagenesis of V823X, internal primers were synthesised and used as described below:

SEQ ID ~~No.57:~~ NO: 57	CGAGGACAGTTATGAAG	(gene nt.2214-2230)
SEQ ID ~~No.58:~~ NO: 58	AGTGGGATCTTCCATCTG	(gene nt.3108-3091)
SEQ ID ~~No.59:~~ NO: 59	ATGTGGGGAGCTACTCATCCC	(gene nt.2523-2503)
SEQ ID ~~No.60:~~ NO: 60	GGGATGAGTAGCTCCCCACAT**gcc**CTAAGAAACAG	(V823A)
SEQ ID ~~No.61:~~ NO: 61	GGGATGAGTAGCTCCCCACAT**tgc**CTAAGAAACAG	(V823C)
SEQ ID ~~No.62:~~ NO: 62	GGGATGAGTAGCTCCCCACAT**gac**CTAAGAAACAG	(V823D)
SEQ ID ~~No.63:~~ NO: 63	GGGATGAGTAGCTCCCCACAT**gag**CTAAGAAACAG	(V823E)
SEQ ID ~~No.64:~~ NO: 64	GGGATGAGTAGCTCCCCACAT**ggg**CTAAGAAACAG	(V823G)
SEQ ID ~~No.65:~~ NO: 65	GGGATGAGTAGCTCCCCACAT**cac**CTAAGAAACAG	(V823H)
SEQ ID ~~No.66:~~ NO: 66	GGGATGAGTAGCTCCCCACAT**aag**CTAAGAAACAG	(V823K)
SEQ ID ~~No.67:~~ NO: 67	GGGATGAGTAGCTCCCCACAT**aac**CTAAGAAACAG	(V823N)
SEQ ID ~~No.68:~~ NO: 68	GGGATGAGTAGCTCCCCACAT**ccc**CTAAGAAACAG	(V823P)
SEQ ID ~~No.69:~~ NO: 69	GGGATGAGTAGCTCCCCACAT**cag**CTAAGAAACAG	(V823Q)
SEQ ID ~~No.70:~~ NO: 70	GGGATGAGTAGCTCCCCACAT**cgc**CTAAGAAACAG	(V823R)
SEQ ID ~~No.71:~~ NO: 71	GGGATGAGTAGCTCCCCACAT**agc**CTAAGAAACAG	(V823S)

SEQ ID ~~No. 72:~~ NO: 72 GGGATGAGTAGCTCCCCACATaccCTAAGAAACAG (V823T)

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Please replace the paragraph beginning at line 32 on page 42 and continuing through line 2 on page 43 of the specification with the following amended paragraph:

-- Comparison of expression levels and activity for the above mutants revealed that a variety of changes could be made without substantially affecting expression levels and activity. These data are depicted in FIGURE 7. Alteration of V823 to A, D, E, G, H, N, P, S or T yielded mutants with expression and activity at least equivalent to that of wt ~~factor~~ Factor VIII (all residue numbering based on SEQ ID NO: 73). Therefore these mutations are useful for the removal of T cell epitopes associated with peptide 7. --

Please replace the paragraph at lines 6-13 on page 43 of the specification with the following amended paragraph:

-- FVIII derived peptides were synthesised containing mutations and tested for their continued ability to promote T-cell proliferation using an ex vivo assay. The peptides were 15mer sequences and were designed to test the substitutions I1011A or I1011T in combination with M1013K (all residue numbering based on SEQ ID NO: 73). The peptides were tested using 4 PBMC donor samples shown previously to be responsive to the wild-type peptide sequence. In all instances the mutant peptides tested were unable to stimulate proliferation with an SI > 2.0. Results of this assay including allotype details of the donors and peptide sequences are shown in FIGURE 8. --